Remarks and Arguments

This response follows a telephone interview between the examiner, the undersigned, inventor Markus Kostrzewa and colleague Karsten Michelmann. During that interview, the parties discussed the application, the claims and the outstanding office action. The amendments above are made to reflect those discussions.

All of the claims of the application currently stand rejected under 35 U.S.C. §103(a) as obvious over U.S. Patent No. 6,221,601 ("Koster '601") in view of either U.S. Patent No. 5,547,835 ("Koster '835") or U.S. Patent No. 5,830,655 ("Monforte"). In making this rejection, the examiner has stated that Koster '601 discloses providing an extension primer having a nucleotide chain that contains a cleavable linker and attaching the primer to DNA adjacent to a mutation site, extending the primer using mutation dependent primer extension, cleaving the linker to produce a DNA cleavage product and analyzing the cleavage product using mass spectrometric analysis. The examiner has stated that Koster '601 does not disclose a photocleavable linker, and therefore cites Koster '835 and Monforte.

In a written response dated July 23, 2004, the applicants argued certain distinctions between the claims of the present application and the cited prior art. In the most recent office action, however, the examiner stated that the applicants arguments did not correspond sufficiently to specific claim limitations.

Koster '601 discloses a method of DNA detection using mass spectrometry. Koster '601 discusses different embodiments for doing the DNA detection, and mentions the use of a cleavable linker located at the 5' end of the primer. The apparent reason for this is to allow the primer and extension products to be released after being immobilized by attachment to a solid support. Cleavage would then allow measurement of the cleaved primer/extension products by mass spectrometry. However, Koster '601 is unconcerned with controlling the length of the fragments for improving their measurement by mass spectrometry.

Koster '601 performs a primer extension that is terminated using a set of four terminating and non-terminating nucleoside triphosphate derivatives. A non-terminating nucleoside triphosphate derivative will be referred to herein by the common nomenclature "dNTP," while a terminating nucleoside triphosphate derivative will be referred as a "ddNTP." Koster '601 puts forth only two ways of performing a primer extension in mutation analysis. The first is to provide a set of four ddNTPs. This, necessarily, requires that the primer is hybridized directly adjacent to the mutation site, since the extension will terminate after a single base extension. This is mentioned, for example, in column 4 line 55 to column 5 line 9 of Koster '601. In another embodiment, Koster '601 uses one ddNTP and three dNTPs (see column 28, line 59 through column 29, line 9). The use of this set of terminating and non-terminating NTPs results in extension products that are not optimal for mass spectrometric analysis. In particular, the mutation dependent mass differences can become so large that the sensitivity for detecting heterozygous alleles is very different resulting in a false mutation diagnosis.

The Koster '835 and Monforte references are discussed in a prior response, and are cited for showing the use of a photocleavable linker. Thus, the discussion herein of the cited prior art combinations focuses primarily on the Koster '601 reference. These discussions, however, are intended to apply to the prior art combinations as a whole.

The present invention is directed to mutation analysis that uses modified primers in a primer extension that is terminated at the same point in all cases for a given target molecule, thereby resulting in fragments all having the same length. The primer includes a photocleavable linker that is positioned within a few nucleotides of the 3' end of the primer. After mutation-dependent extension of the primer, the linker is cleaved and short fragments of the extended primer are generated which serve as products for the mass spectrometric measurements. These short fragments contain the desired information regarding the mutation. The length of all the fragments derived from one reaction is the same. The reaction products for different alleles differ in their molecular weight depending on the kind of mutation. The determination of the mass of all fragments with the same length yields the correct genotype which has to be determined.

Unlike the Koster '601 prior art, the present invention makes use of sets of terminating and non-terminating NTPs that include three ddNTPs and one dNTP, or ever two ddNTPs and two dNTPs. Functionally, the applicants' invention gains certain advantages with the use of these ratios, as compared to the ratios used in Koster '601. The use of three ddNTPs rather than four allows the primer to be attached at a position other than directly adjacent to the mutation site. The applicants have found that attaching a primer with a photocleavable linker directly adjacent to the mutation site can result in molecules tending to fold over on themselves, which seriously distorts the ultimate mass measurement. By attaching further from the mutation site, this can be avoided. Similarly, the use of two or three ddNTPs is preferable to the use of just one, since the use of a single ddNTP can lead to the termination of two extension products being at positions very far from each other. In such a case, an extension product that is produced when the mutation is present may be vastly different in length than an extension product produced when the mutation is absent. This great disparity in length creates a great disparity in the masses of the two molecules and, when detected by mass spectrometry, can result in a reduction in the sensitivity of the overall mass spectrum due to the need to examine a much wider mass range. By using more ddNTPs, there are more termination sites, and the resulting molecules are closer in length and, therefore, closer in mass.

In addition to the difference in the makeup of the dNTP/ddNTP sets, the present invention also allows for the use of sets that are less than "complete," that is, which have fewer than four total dNTPs and ddNTPs. While Koster '601 does not contemplate such a method, the applicants have found that it is possible to do so, due to the fact that they are looking for a known mutation on a known molecule, and are producing short extension segments. Therefore, it is quite possible that the production of a useful extension segment does not require all four base types, and a reduced set of dNTPs and ddNTPs may therefore be used. In addition to simplifying the reaction components, and reducing the risk of an incorrect base type being accidentally hybridized in a particular location, the use of a reduced set also decreases the cost of the reaction, since fewer components must be used.

The claims of the application have been amended in an attempt to better highlight the distinctions discussed above. In particular, Claim 1 has been amended to specify that the step of extending the primer is done using a complementary mixture of non-terminating and terminating nucleoside triphosphate derivatives, where the mixture has "a ratio of one non-terminating to three terminating nucleoside triphosphate derivatives, or two non-terminating to two terminating nucleoside triphosphate derivatives." Such a method is not suggested by the cited prior art combinations, and provides distinct advantages, as described above. Claim 6 has been canceled as its limitations have been incorporated into Claim 1 by amendment. Each of Claims 2-5 and 7- 23 depends ultimately from Claim 1 and is therefore equally unsuggested by the cited prior art. Reconsideration of Claims 1-5 and 7-23 under this ground for rejection is respectfully requested.

Since the amendment of Claim 1 narrows the claim to exclude the use of a reduced set of NTPs, that aspect of the invention has been transferred from Claim 1 to a new independent Claim 24. Likewise, Claim 4 has been amended to avoid any confusion in this regard. New Claim 24 is similar to Claim 1 in reciting a method for mass-spectrometric analysis of a known mutation site in genome DNA, but has a different step regarding the primer extension. In Claim 24, extending the primer is done using "a complementary mixture of fewer than four non-terminating and terminating nucleoside triphosphate derivatives." As discussed above, this has particular benefit for this specific type of method, and is not suggested by the cited prior art combinations. New Claims 25-29 repeat some of the limitations of the claims dependent on Claim 1 so as to provide a more complete coverage of this aspect of the invention. Acceptance and allowance of Claims 24-29 is respectfully requested.

In light of the amendments and arguments herein, and those points discussed during the recent telephone interview, Claims 1-5 and 7-29 are believed to be in condition for allowance. If there are any questions or comments regarding these points, the examiner is respectfully requested to contact the undersigned. The Commissioner

is hereby authorized to charge any additional fees due for the filing of this paper to the applicants' attorneys Deposit Account No. 02-3038.

Respectfully submitted

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